

THE COMPONENTS AND COLLOIDAL BEHAVIOR OF PLANT PROTOPLASM.

By D. T. MACDOUGAL AND H. A. SPOEHR.

(*Read April 23, 1920.*)

SUMMARY OF GENERALIZATIONS PREVIOUSLY DISCUSSED.

The principal conclusions established by our previously described investigations which are of direct interest with relation to new results to be presented are as follows:

I. The protoplasmic mass of the active cell of the plant is a mixture of carbohydrates chiefly in the form of pentosans and albuminous substances, with a probable very low but undetermined proportion of lipins. In addition to the mucilaginous substances of the first, freely soluble sugars may be present in the cell solutions.

II. The principal components of plasmatic masses, the mucilages and the proteins, are mutually non-interdiffusible and hence when brought together in the cell by minute accretions or mixed in liquid form must be taken to form complex emulsions or mesh-works, and to occur separately both in disperse phase and disperse medium.

III. Of the components of such a mass the one which could be regarded as the more solid as having the lesser attraction for molecules of water, would tend to take position in the peripheral layer and to assume a greater density by lessening the liquid phase in the surface layer.

IV. The external layer of any colloidal mass or of any layer where two masses meet has invariably a composition determined by the constitution of the impinging masses. The formation of the cellulose wall which is first seen as a free plate between two separating protoplasts has a structure resulting from such action. The plasma of the plant being highly carbohydrate, the external layer is consequently largely anhydride of this material. The layers added internally to the initial wall must be of the same character. Furthermore for similar reasons, the external layer of the plasma, the semi-permeable membrane, would also be high in carbohydrate. The en-

closing and boundary layers of nuclei and of all special bodies in the protoplasm would have a similar dual origin.

V. Highly proteinaceous plasmas would form external layers, which in conformity with the above, would not be cellulose or so high in carbohydrate. The chitinous skins of some animal organisms offer an inviting subject for examination in this connection.

VI. In so far as these limiting layers offer resistance to the passage of substances in solution equally in both directions, or as they allow the free passage of water and resistance to substances in solution, they form an osmotic machine by the action of which pressures may be set up internal to the cell and to plasmatic or nuclear masses. The implied phenomena designated as turgidity are most marked in plant cells where distentive forces of 40 or 50 atmospheres are found. It is to be noted also that when the two elements of a plasmatic colloid, the carbohydrate and the albumin, are unequally hydrated, as is the case in nearly all solutions, the superior increase of one element in the complex meshwork would set up something akin to osmotic pressure.

VII. Hydration increases or swelling is the result of the combination of molecules of water with colloidal aggregates of the mass. The addition of any substance which forms combinations with the colloidal carbohydrate or protein may give systems which attract, combine with and hold proportions of water different from those displayed when water only is present.

VIII. The hydration increase or swelling of an intermeshed pentosan-protein colloid, such as we imagine protoplasm to be, involves the possibility of the unequal increase of these two main components under the influence of any substance or ion, and the measurable alterations in volume will be the resultant of the effects of such a substance or ion upon the hydration of the unlike components.

IX. The pentosans are weak acids and in general their hydration capacity is lessened by hydrogen ions. Hydroxyl ions and compounds containing the amino-groups, such as may be in solutions of phenyl-alanin, alanin, asparagin and glyocoll, may exert an effect by which hydration capacity is increased above that in pure water. Mucilages derived from various sources show some differences in reactions to the solutions named while conforming to the generaliza-

tions given. Their hydration is but little affected by the presence of the common sugars in the water of suspension or dispersion.

X. The albumins and their derivatives are amphoteric, being capable of dissociating both as acids or as bases. Hydration in the presence of hydrogen ions may be much greater than in water and may reach the possible maximum, while that in the presence of hydroxyl ions and of various cations may also be in excess of that in water. Gelatine as an example of this group shows such behavior but has a restricted hydration capacity in amino-acids such as glycocoll. On the other hand, the swelling is proportional to the high hydrogen ion concentration of such amino-acids as aspartic acid, which is diabasic.

XI. Variations in the hydration total or volume may be ascribed to changes in the colloidal components of a plasma, to products of the resident metabolism, to the action of substances absorbed during hydration or to fluctuations in temperature.

XII. The changes in volume of a mass of colloidal material are usually not iso-diametrical during hydration. Such alterations are determined by the structure of the jelly which may be so differentiated as to show expansion and contraction along one axis almost wholly.

XIII. The analysis of the implied facts has also demonstrated that growth is so essentially as to its nature, and so largely as to volume, a matter of hydration that the compounds which facilitate the swelling of phytocolloids and of cell-masses, facilitate or accelerate growth.¹

¹ MacDougal, D. T., "Hydration and Growth," Publ. 297 Carnegie Inst. of Wash., 1920, and "Hydration Effects of Amino-compounds," *Proc. Soc. for Exper. Biol. and Med.*, 17: 35-36. 1919.

Schreiner, O., and Skinner, J. J., "Experimental Study of the Effect of Some Nitrogenous Soil Constituents on Growth. Nucleic Acid and Its Decomposition Products," *Plant World*, 16: 45-60. 1913.

Schreiner, O., and Skinner, J. J., "Specific Action of Organic Compounds in Modifying Plant Characteristics; Methyl Glycocoll versus Glycocoll," *Bot. Gaz.*, 59: 445-463. 1915.

Skinner, J. J., and Beattie, J. H., "Effect of Asparagin on Absorption and Growth in Wheat," *Bull. Torr. Bot. Club*, 39: 429-437. 1919.

Borovicow, G. A., "On the Action of Different Substances on the Velocity of Growth of Vegetables," Publ. of the Soc. Nat. of New Russia, 41: 15-194. 1916.

CONCLUSIONS FOUNDED ON NEWLY OBTAINED RESULTS.

The further development of our knowledge on this subject has been attempted by experiments arranged to obtain evidence upon four topics, viz: (1) the proportions of carbohydrate and albuminous matter in a colloid of the highest hydration capacity; (2) the substances or ions of biological significance which would raise the hydration capacity of these phytocolloids to the highest limit; (3) measurement of the relative effects of some metallic bases upon a carbohydrate colloid; and (4) determination of the amplitude and continuance of alternating or repeated effects of renewed or replaced solutions.

1. By the use of the pentosan, agar, as representing the acid carbohydrate and of gelatine for the amphoteric albuminous component trials were made to ascertain what proportions of these substances would show hydration capacities of a range comparable to that of living matter. A mixture containing one part carbohydrate and three parts albuminous matter shows the-highest general hydration capacity under the influence of hydrogen, hydroxyl ions and the ions which may be derived from amino-acids. Biocolloids high in albuminous matter swell most under the action of the hydrogen ion. Biocolloids containing 40 per cent. or more carbohydrates swell most in amino-compounds. Balanced biocolloids swell most in the presence of hydroxyl ions. These reactions are parallel to those of living and dried cell-masses of plants, and follow through the seasonal variations determined by chemical analyses.

2. Biocolloids of which more than a fourth is carbohydrate are highly sensitive to the action of hydrogen ions, which restrict hydration.

3. The basic histidine and glycocoll which is slightly on the acid side of neutral increase hydration in biocolloids containing more than 40 per cent. carbohydrate. Maximum swellings of 4300 per cent. by a mixture of 1 part agar and 3 parts gelatine in acid representing a high concentration for plant juices, and of 3930 per cent. by a mixture of 2 parts agar and 3 of gelatine in histidine are of great physiological interest. But little information concerning the presence or action of the basic amino-compounds in plants is available.

4. Glycocoll and glycocoll ester increase the swelling of agar. Glycocoll lessens swelling of gelatine, while glycocoll ester, glycocoll ester hydrochloride and glycocoll hydrochloride increase it beyond that shown in distilled water.

5. The hydroxides of the strong metallic bases limit the hydration of agar according to their position in the electromotive series, the least swelling taking place under the action of the strongest base at concentrations of 0.01 *N* with the apparent exception of rubidium. Beginning with the strongest the series runs K (Rb) Na Li.

6. The various effects of barium, calcium and strontium are not so clearly determined and the quantitative relations of these metals are not known definitely. Hydration values of agar at 0.01 *N* were $\text{Sr}(\text{OH})_2 = 815$, $\text{Ca}(\text{OH})_2 = 860$, $\text{Ba}(\text{OH})_2 = 900$.

7. Hydration of agar in calcium hydroxide exceeds that in water at 0.0001 *N* of the hydroxide and this effect is also produced at 0.00001 *N*. Increase of hydration beyond that of water by dilute solutions of hydroxides of calcium, potassium, rubidium, potassium sodium and lithium is an effect we have hitherto ascribed to amino-compounds only. Excess values for aniline and ammonium hydroxide are given.

8. The incorporation of bases in agar lessens its hydration capacity in any concentration yet tested, and this is also true of biocolloids of which carbohydrates constitute more than half. In mixtures containing more gelatine hydration capacity in acids and in hydroxides may be increased by included bases. The inclusion of a metallic base and its presentation in a hydrating solution would give different results in a colloidal or plasmatic body such as a nucleus or chromosome.

9. The data in this article were secured chiefly by the swelling of trios of sections with a total volume of 4 to 8 cu. mm. under the auxograph in dishes into which 25 to 30 cc. of solution was placed and renewed at intervals of 12 and 24 hours. Such renewals were attended by accelerations in the rate and increases in the total swelling. Agar and biocolloids of agar and gelatine showed this action in a marked manner. Sections of equal parts of these two components exhibited reactions in which the exaggerated swelling resulting from renewals were partly retracted very slowly on the third

day. After this the exaggeration slowly decreased and the retraction increased until the two balanced about the eighth day. The two movements continued for a total period of 67 days. It is suggested that the exaggerated swelling following a renewal of the solutions may be due to the formation of glycocoll agarate, the bulk of which might be greater than that of the agar. Diffusion of this material from the section would result in a retraction or shrinkage.

10. Plates of colloids cast on glass and prevented from shrinking in area take on a heterotropic structure which varies in agar, gelatine and in mixtures of the two. The swelling of an agar plate is almost wholly in thickness so that the increase of a hydrated section is denoted directly by the thickness reached. Gelatine plates prepared in the same manner may increase as much as 60 per cent. in length and width while swelling. Plates of mixtures of the two swell from 6 to 16 per cent. in length and width, this amount being modified by the character of the hydrating solution. These effects which may play an important part in morphological procedure in the cell, seem to indicate a meshwork structure of biocolloids as it does not seem possible for emulsions to be differentiated in the manner implied.

HYDRATION TESTS OF VARIOUS BIOCOLLOIDS.

In the effort to ascertain the character of the biocolloids which might show hydration reactions of the range and variety of protoplasm, empirical mixtures of agar and gelatine were made up and cast into plates which were dried and then swelled under the auxograph. Eight colloidal preparations were hydrated in water, hydrochloric acid, potassium hydroxide and glycocoll at 15° C. Trios of sections were swelled under the auxograph in the usual manner, the increases being calculated in percentages of original thickness by the left hand number of each couple. Strips of the same material 50 to 80 mm. in length were placed in test tubes of similar solutions. The resulting increases ranged from 2 to 12 per cent. in length and width in the different colloids. These ratios were applied to the increases in thickness to obtain the total volumes expressed in the right-hand number of each pair in Table I.

TABLE I.

HYDRATION OF AGAR, AGAR-ALBUMIN, AGAR-GELATINE AND GELATINE IN TERMS OF THICKNESS AND VOLUME.

HCl, 0.01 N.		KOH, 0.01 N.		Glycocoll, 0.01 M.		Water.	
Th.	Vol.	Th.	Vol.	Th.	Vol.	Th.	Vol.
<i>Agar 0.16 mm. at 15° C. Compiled Data.</i>							
800	1,000	600	750	3,300	3,500	1,800	2,000
<i>Gelatine 0.25 mm. at 15° C.</i>							
1,960	4,700	1,640	3,190	600	850	960	1,570
<i>Agar 1, Gelatine 3, 0.18 mm. 12-15° C.</i>							
2,500	4,300	2,500	3,600	2,460	2,975	1,445	1,660
<i>Agar 2, Gelatine 3, 0.19 mm 15° C.</i>							
1,580	1,808	2,380	3,375	3,050	3,690	1,980	2,255
<i>Agar 3, Gelatine 3, 0.25-28 mm. 14-16° C.</i>							
1,300	1,573	2,720	3,802	2,200	2,565	1,860	2,250
<i>Agar 3, Gelatine 2, 0.18 mm. 14-16° C.</i>							
900	964	2,556	3,090	3,220	3,416	3,000	3,245

The data in the above table afford information on three main questions, viz., the probable constitution of living matter to be inferred from high hydration capacity, the nature of the colloids which show a sensitiveness in hydration to the action of the hydrogen ion, the hydroxyl ion and to ions which may be derived from amino-compounds, and lastly the differentiations in heterotropic swelling.

The highest swelling of the pentosan-protein mixtures is that of agar 1 gelatine 3 in hundredth normal acid. Such colloids may be present in the animal, but may be taken to be highly specialized or unusual in the plant and not shown by the cell-masses of the vegetative tracts. Chief interest centers in the colloids in which the carbohydrate and albuminous components each vary in forming 30 to 60 per cent. of the total and showing high hydration capacities in the hydroxides, in glycocoll and in water. These mixtures furnish an analogue of living matter of proved similarity of composition and action. Not only has it been possible to compound biocolloids which

would furnish conditions parallel to those in the plant, but the influence of changing proportions of the pentosans to the other cell-contents has been followed through the season by analyses in the chemical laboratory and by swelling tests of living and dried sections of the plants.²

Next to the composition and to the condition of the components, the matter of greatest importance is that of the ions or substances which may determine the course and amount of hydration. Acids, hydroxides and amino-compounds are to be included in a list of the substances of physiological importance. The action of hydrogen ions, of hydroxyl ions and of ions which may be derived from amino-compounds in the way of accelerating or retarding hydration may be shown by expressing the swelling values produced in terms of those obtained in water taken as 100 given in Table II.

TABLE II.

HYDRATION OF COLLOIDS IN HYDROCHLORIC ACID, POTASSIUM HYDROXID AND GLYCOCOLL AT 0.01 N WITH THAT IN WATER AS 100.

Hydrochloric Acid.		Potassium Hydroxide.		Glycocoll.	
Th.	Vol.	Th.	Vol.	Th.	Vol.
<i>Agar.</i>					
44	50	33	42	183	185
<i>Gelatine.</i>					
204	300	171	200	63	54
<i>Agar 1 Gelatine 3.</i>					
173	270	173	225	170	186
<i>Agar 2 Gelatine 3.</i>					
80	67	120	150	154	163
<i>Agar 3 Gelatine 3.</i>					
70	70	146	170	112	114
<i>Agar 3 Gelatine 2.</i>					
30	30	85	95	107	106

² MacDougal, D. T., "Hydration and Growth," Publ. 297 Carnegie Inst. of Wash., 1920, p. 132.

The effect of the hydrogen ions which are present in a concentration of $\text{pH}=3$ in the acid solution is to induce a high swelling in gelatine and to produce a lessened swelling as this component is lessened and as the carbohydrate is increased. A finely parallel series was obtained in tests of living sections of *Opuntia* from last December to March in which the proportionate swelling in acid varied from 80 per cent. in December to 77 in January, 7 in February, rising to 78 in March and falling to 64 in April, as compared with water at 100. The course of the pentosans was not followed at the same time, but in a previous year the variation in the pentosan content of similar material was from 10 in late December to 4.7 in mid-January, 6 in mid-February, and to 5.5 late in March, which allowing for seasonal differences, gives a fair parallel.

The hydroxide is seen to cause a swelling of both agar and of gelatine less than in water, and to cause a swelling of gelatine something less than in acid. Its general effect is to lessen hydration as the carbohydrate component of the biocolloid becomes greater, although an aberrant high swelling is shown by mixtures of equal parts of gelatine and agar.

Turning now to the plant material which shows the seasonal variation of pentosans noted above the swelling of living material varies from comparative values of 100 in December, 103 in January, 100 in February, and 110 in March, facts in no wise discordant with the seasonal changes and probable accumulation of metallic salts in the cells.

Glycocoll produces a maximum effect on agar and a minimum on gelatine or the albuminous component of biocolloids. Its maximum accelerating effect seems to be upon mixtures containing 25 to 40 per cent. of the carbohydrate, although in all cases it causes agar mixtures to hydrate to a point beyond that which might be reached in water.

If we now seek to ascertain what type of biocolloid is capable of the greatest average hydration or growth under the influence of these substances and of the basic histidine, it will be found in a mixture which is composed of 1 part carbohydrate and 3 parts albuminous material. Biocolloids containing more carbohydrate than albuminous matter would be most sensitive to the presence of hy-

drogen ions, and their growth would be markedly limited by acidity. Such mixtures would also be modified something less by hydroxyl ions. All types of biocolloid would respond by increased hydration to the presence of amino acids as shown by the relative swellings in glycocoll and in histidine.

The results with the basic histidine and of its salt histidine dihydrochloride, which reacts as an acid, are as given in Table III.

TABLE III.

SWELLINGS OF AGAR, GELATINE AND MIXTURES IN PERCENTAGES OF ORIGINAL THICKNESS AND VOLUME.

Histidine, 0.01 M.		Histidine Dihydrochloride, 0.01 M.		Water,	
Th.	Vol.	Th.	Vol.	Th.	Vol.
<i>Agar 0.15 mm. at 15° C.</i>					
2,500	900	3,100
<i>Gelatine 0.28 mm. at 14-16° C.</i>					
1,723	3,103	1,600	4,200	1,400	3,150
<i>Agar 2 Gelatine 3, 0.19 mm. at 15° C.</i>					
3,470	3,930	1,445	1,780	1,980	2,225
<i>Agar 3 Gelatine 3, 0.25 mm. at 15° C.</i>					
....	1,200	1,320	1,860	2,250
<i>Agar 3 Gelatine 2, 0.25 mm. at 14-16° C.</i>					
3,472	3,755	800	842	3,000	3,245

Mixtures consisting of 40 to 60 per cent. of the two main components are seen to give the highest swelling values in histidine yet obtained by biocolloids by treatment with any reagent. Furthermore this amino-compound acts to increase the swelling of gelatine and all mixtures containing it to a point beyond that which is possible in water. Its acid salt has the well-known effect on agar, gelatine and their mixtures. These features are illustrated by Table IV. in which the values are given in terms of water as 100.

In addition to the high hydration caused in the above mixtures by histidine, attention has been previously called to a similar action

by glycocoll on agar. No final explanation for this behavior has as yet been obtained. Of great interest, however, are the results obtained with a number of glycocoll compounds. Swelling tests were made in the usual manner with the following compounds: (1) Glycocoll which is approximately neutral in reaction and amphoteric in behavior. (2) Glycocoll ethyl ester; in this compound the acid radicle has been neutralized, is distinctly alkaline and acts as a weak

TABLE IV.

Histidine.		Histidine Dihydrochloride.	
Th.	Vol.	Th.	Vol.
<i>Agar.</i>			
80	30
<i>Gelatine.</i>			
124	100	114	130
<i>Agar 2 Gelatine 3.</i>			
176	176	73	80
<i>Agar 3 Gelatine 3.</i>			
....	65	60
<i>Agar 3 Gelatine 2.</i>			
116	116	27	27

base on the swelling of agar. However, in solutions of this substance agar does not attain the same swelling above that of water as it does in the amphoteric glycocoll. On account of the relatively rapid hydrolysis of glycocoll ester in water, fresh solutions were frequently prepared for renewal in the swelling tests. (3) Glycocoll ethyl ester hydrochloride in which both the basic and acid portions of the glycocoll molecule have been neutralized. (4) Glycocoll hydrochloride which reacts as an acid in water solution and shows the typical acid behavior in the swelling agar. The solutions were all in 0.01 molar concentration. The absolute increases in terms of thickness and volume are as below:

TABLE V.

Water.	Glycocoll 0.01 M.	Glycocoll Ester 0.01 M.	Glycocoll Ester Hy- drochloride 0.01 M.	Glycocoll Hydro- chloride 0.01 M.					
<i>Agar.</i>									
Sections 0.15 mm. Thick at 15° C.									
3,220	4,130	3,360	1,530	1,000					
(Increases in width and length very slight.)									
<i>Gelatine.</i>									
Sections 0.23 mm. Thick at 15° C.									
Th.	Vol.	Th.	Vol.	Th.	Vol.	Th.	Vol.	Th.	Vol.
960	1,570	600	850	1,170	2,240	910	1,180	1,280	2,880

The above data reduced to terms of swelling in water as 100 give values as in Table VI.

TABLE VI.
SWELLING OF DRIED PLATES OF AGAR AND GELATINE.

Water.		Glycocoll.		Glycocoll Ester.		Glycocoll Ester Hydrochloride.		Glycocoll Hydrochloride.	
Agar.									
100		128.30		104.35		47.50		31.07	
Gelatine.									
Th.	Vol.	Th.	Vol.	Th.	Vol.	Th.	Vol.	Th.	Vol.
100	100	62	54	122	143	95	75	134	180

Glycocoll and its ester are seen to increase the swelling of agar and to lessen that of gelatine. The two salts exert the classical effects of retarding the swelling of agar. The swelling of gelatine in the glycocoll ester hydrochloride is slightly less than in water, while the swelling of gelatine in the glycocoll ester hydrochloride is higher as in an acid solution.

HYDRATION OF AGAR IN SOLUTIONS OF VARIOUS HYDROXIDES.

The delicacy of reaction prevailing in the behavior of agar towards hydrogen and hydroxyl ions as well as various cations is

exemplified in the swellings in solutions of various bases and derivatives of glycocoll. It became apparent in the earlier stages of our work that the effects of the alkaline hydroxides were by no means equivalent, and a series of preparations were run for the purpose of securing comparisons of the action of potassium, rubidium, sodium, lithium, barium, strontium and calcium. The values obtained are given in Table VII.

TABLE VII.
SWELLING OF AGAR IN STRONG ALKALINE HYDROXIDES.
Sections 0.14 mm. Thick at 17° C.

	0.01 N.	0.001 N.
KOH	1,535	3,430
RbOH	1,635	3,500
NaOH	1,645	3,430
LiOH	1,820	3,430
Water	3,000 to 3,070	

When these absolute values are compared with that obtained in water the data in Table VIII. are obtained.

TABLE VIII.
SWELLING OF DRIED AGAR PLATES 0.14 MM. IN THICKNESS AT 17° C. IN SOLUTIONS OF ALKALINE HYDROXIDES WHICH WERE RENEWED EVERY 12 HOURS.
Total swelling of dried agar plates in water 3,035 per cent.

Normal Concentration.	Water.	KOH.	RbOH.	NaOH.	LiOH.
0.01	100	50.3	53.8	54.8	60.0
0.001	100	113.0	115.3	113.0	113.0

It is to be seen that these alkaline hydroxides may be carried to an attenuation where they may cause a swelling of agar greater than in water, an effect hitherto found only with amino-compounds. The stronger the base as indicated by its position in the electromotive series, the more does it restrict hydration. All reverse that effect and become accelerating agents at a greater dilution. Rubidium is the exception in the table, and its aberrant position may be ascribed to error until material is available for a repetition of the tests.

In the hydroxides of the alkaline earths the variations are not so clearly defined. Unfortunately the quantitative relations of these

metals are not definitely known, and further complications may arise in the different solubility and behavior of the carbonates which naturally are always formed in spite of frequent renewals of the solutions. The series of tests which were made to secure information on this matter yielded the following results:

TABLE IX.

SWELLING OF AGAR IN ALKALINE HYDROXIDES IN PERCENTAGES OF ORIGINAL THICKNESS.

	0.01 <i>N.</i>	0.001 <i>N.</i>	0.0001 <i>N.</i>	0.00001 <i>N.</i>
Sections 0.14 mm. Thick at 15° C.				
Ba(OH) ₂	900	1,145	2,430	2,400
Sections 0.14 mm. Thick at 15° C.				
Ca(OH) ₂	860	1,220	3,200	3,200
Sections 0.16 mm. Thick at 15° C.				
Sr(OH) ₂	815	1,565	2,565	2,665

The conversion of the data in Table IX. to terms of swelling in water results in the following data:

TABLE X.

SWELLING OF DRIED AGAR PLATES AT 15° C. IN SOLUTIONS OF ALKALINE EARTH HYDROXIDES WHICH WERE RENEWED EVERY 12 HOURS.

Thickness of dried plates in Ba(OH)₂ and Ca(OH)₂ series 0.14 mm., in Sr(OH)₂ series 0.16 mm. Total swelling in water 3,035 per cent.

Normal Concentration.	Water.	Ba(OH) ₂ .	Sr(OH) ₂ .	Ca(OH) ₂ .
0.01	100	29.6	26.8	28.3
0.001	100	47.7	51.6	40.2
0.0001	100	80.3	84.8	106.0
0.00001	100	79.4	88.0	106.0

It is seen that even at the greater attenuations, barium and strontium hydroxides limited hydration. As it appeared important to test the favorable effect of calcium solutions of various concentrations, a detailed series of tests was carried out. The actual increases in percentages of original thickness are given in the upper line of the table below and the values as compared with water as 100 in the line below.

TABLE XI.

SWELLING OF DRIED AGAR PLATES AT 15° C. IN SOLUTIONS OF $\text{Ca}(\text{OH})_2$ WHICH WERE RENEWED EVERY 12 HOURS.

Normal Concentration.	Water.	0.02.	0.01.	0.002.	0.001.	0.0002.	0.0001.	0.00001.
	3,000	565	860	1,000	1,220	2,500	3,200	3,200
	3,070							
	100	18.6	28.5	33.0	40.2	82.4	105.4	105.4

The augmenting effect of calcium hydroxide which was found in concentrations of 0.0001 is here seen not to be exhibited in a solution containing twice this amount, and is not increased when a reduction below this concentration is made.

The effects of ammonium hydroxide and aniline on the swelling of agar are of direct interest in any discussion of the relative action of the weaker and stronger bases.

Data for comparisons have been conveniently grouped in Table XII.

TABLE XII.

SWELLING OF DRIED AGAR PLATES AT 15° C. IN SOLUTIONS OF VARIOUS HYDROXIDES, RENEWED EVERY 12 HOURS, IN TERMS OF WATER AS 100.

Total swelling of dried agar plates in water 3,950 per cent.

Normal Concentration.	Water.	Aniline.	Ammonium Hydroxide.	Ethylamine.	Lithium Hydroxide.	Sodium Hydroxide.	Potassium Hydroxide.
0.01	100	110	25	31	24	21	21
0.001	100	100	115	88	40	35	29

The behavior of agar in the weak bases ammonium hydroxide, ethylamine and aniline on the one hand and in lithium hydroxide, sodium hydroxide and potassium hydroxide on the other, exhibits some interesting differences, particularly in the more dilute solutions.³ Owing to the fact that in solutions of ammonium hydroxide and ethylamine there exist equilibria respectively between dissolved NH_3 and the hydroxide, and between dissolved $\text{C}_2\text{H}_5\text{NH}_2$ and its hydroxide, the condition in solutions of these substances particularly in the more concentrated solutions offer a rather complicated situa-

³ MacDougal, D. T., and Spoehr, H. A., "The Swelling of Agar in Solutions of Amino Acids and Some Related Compounds," *Bot. Gaz.*, 69: 1920 (in press).

tion not very dissimilar from that obtaining in solutions of the amino acids. It will be recalled that aniline is a weaker base than ammonium hydroxide while ethylamine and the hydroxides of lithium, sodium and potassium are stronger.

EFFECT OF INCLUDED BASES ON SWELLING OF BIOCOLLOIDS.

The hydration of plates or sections of dried colloids in solutions involves questions of penetration and of the formation of compounds in the external parts of the section which may modify the hydration of the interior of the mass. The conclusion was reached in work set forth in previous papers that acids, metallic salts and

TABLE XIII.

SWELLINGS OF DRIED SECTIONS OF BICOLLOIDS WITH INCLUDED BASES IN PERCENTAGES OF ORIGINAL THICKNESS.

HCl 0.01 N.	KOH 0.01 N.	Histidine Dihydrochloride 0.01 N.	Glycocoll 0.01 N.	Water.
Agar 2, Gelatine 3, KOH 0.000,05 N NaOH 0.000,025 N Ca(OH) ₂ 0.000,025 N Sections 0.1 mm. Thick at 16-17° C.				
2,100-3,220	2,950-3,570	1,700-2,050	2,750-3,030	2,050-2,380
Agar 3, Gelatine 3, KOH 0.001 N NaOH 0.0001 N Ca(OH) ₂ 0.0001 N Histidine 0.001,4 Section 0.09 mm. Thick at 16-17° C.				
850-875	2,220-2,680	1,700-1,800		2,050-2,260
Agar 3, Gelatine 2, KOH 0.000,05 N NaOH 0.000,025 N Ca(OH) ₂ 0.000,025 N Sections 0.14-0.15 mm. Thick at 15° C.				
500-521	2,330-2,820	1,570-1,730	2,850-3,200	2,300-2,780

amino-compounds incorporated in colloids exerted a greater effect, generally a lessening action on swelling than the same amount of the reagent applied in aqueous solution.

Furthermore it may be said in particular that in no case did an

inclusion in the carbohydrate, agar, increase its hydration in water, and this holds true for such substances as asparagin, glyocoll, etc., which cause a hydration much in excess of that taking place in water when applied in aqueous solution.

As a further contribution to this matter the hydroxides of calcium, sodium and potassium were incorporated in biocolloids in proportions in which no lessening effect would be exerted by solutions which would bring the same amount of the bases into action on the colloids. The results of this series of tests are given in Table XIII.

The hydration values of the mixtures of agar 2 parts and gelatine 3 parts in water are not materially different from those of some colloidal mixture free from the bases. The presence of the bases increases hydration in acid and in potassium hydroxide, but lessens it in glyocoll, as may be seen by comparison with Table I. When the proportion of agar is increased and that of gelatine decreased as in a mixture of 3 parts agar and 2 of gelatine, the swelling in water is lessened notably, and decreases occur in all solutions, a fact which may be ascribed directly to the action of the carbohydrate component. The bases included in these biocolloids were present in proportions one fourth of that in which excess swelling was caused in solutions of calcium and sodium, and one five hundredth in the case of potassium, although the total amount of bases would be little short of that present in any one of the solutions.

It is suggested that the reversal of the effect of included bases on the swelling of biocolloids must be at a greater attenuation than when in solution. Such restricting effect rises with the proportion of carbohydrate present.

It is evident that the inclusion of a substance or ion in a colloidal structure results in hydration relations of a different character from those which appear when the substance in question is presented in the hydrating solution. In the latter case it seems theoretically possible that differentiations of external layers of the hydrating masses may take place which might result in swellings due in the last analysis to something like osmosis.⁴

⁴ MacDougal, D. T., "Hydration and Growth," Publ. No. 297 Carnegie Inst. of Wash., 1920, see pp. 32, 33, 44, 46, 47, 48, 50, 58, 59, 70, 72, 75.

The presence of any substance in the nucleus, chromosomes or plasmatic bodies would give colloidal reactions materially different from those to be expected when such substances are presented in the cell sap or fluids according to the facts presented above.

MAXIMUM EFFECTS PRODUCED BY RENEWING SOLUTIONS.

Closely related to the effects resulting by replacement of one solution by another are those produced by renewing solutions. The methods of auxographic measurement which have been used so extensively in these experiments entail the immersion of a trio of dried sections of a total volume of about 4 to 8 cu. mm. in 25 to 30 cc. of solution, the most common concentration of which was 0.01 *N*. The procedure of drawing off this solution and replacing it with a fresh solution at intervals of 12 or 24 hours has been followed since 1918.

It has been noted that at the first renewal made after the experiment has been started an acceleration of the swelling would ensue. This speeding up has been attributed to two causes. First, as much as 10 per cent. of the colloid may be drawn out into solution and the presence of such a solution around the mass would operate to lessen the rate of absorption of water by the more solid sections. Secondly, the ions of the substances in the hydrating solution pass into the colloidal mass and enter into combination with the aggregates, thereby lessening the concentration of the solution and consequently its accelerating effect on hydration. The renewal of the solution would remove the colloidal suspension about the sections and would furnish a solution capable of exerting a hydrating effect equivalent to the original, which would speed up the absorption of water.⁵

Usually these effects are shown during the first two or three changes in swellings in which the total effect is practically finished in ten days at 15° C.

Dried sections of agar and a number of mixtures of agar and gelatine are seen to show a much more pronounced and long continued reaction of this kind. The characteristic effect has been produced so far only by attenuated solutions of hydroxides of weak metallic bases, by glycocoll and the basic glycocoll ester.

⁵ MacDougal, D. T., and Spoehr, H. A., *Bot. Gaz.*, 69: (in press). 1920.

Some mention of this action of glycocoll has already been made in a previous article, but the effects shown by agar-gelatine mixtures in which the two principal components vary between 40 and 60 per cent. were so marked that attention was again directed to their measurement and to the formulation of some explanation.

The most pronounced effects were secured by hydrating sections of equal parts gelatine and agar, 0.25 mm. in thickness at 14–16° C., which approximated a condition of saturation in ten days with an increase of 2,640 per cent. in thickness and of 2,720 per cent. in volume, the material being highly heterotropic.

The first renewal of the solution caused a sudden enlargement which in 3 hours added 140 per cent. to the thickness of the sections. The accelerated enlargement following the second change was about 120 per cent., the sections coming to rest on the first day in 4 hours and in 3 hours on the second change. The accelerated swelling on the third day amounted to 100 per cent., coming to rest in something over 2 hours, after which a slow shrinkage occurred by which a third of the previously gained amount was lost. The fourth acceleration amounted to 120 per cent. with a subsequent loss of one third this amount in the next twenty-four hours. The fifth reaction gave a swelling of 100 per cent. followed by a loss of half this amount. The sixth reaction gave an increase of 100 per cent. followed by a loss of equal amount. The seventh reaction gave an increase of 90 per cent., followed by a loss of 50 per cent. The eighth showed a gain of 80 per cent. and a loss of equal amount; the ninth a gain of 70 per cent. and a loss of equal amount; the tenth a gain of 60 per cent. and a loss of 60 per cent.; the eleventh a gain of 80 per cent. and a loss of 60 per cent. The twelfth change gave a gain of 60 per cent. and a loss of 50 per cent.; the thirteenth a balanced loss and gain of 60 per cent., after which the change in both directions became equivalent so that on the sixtieth day the alteration amounted to 40 per cent. of the original thickness, which decreased to 20 per cent. a week later when the observation was closed, 67 days from the beginning. The whole series of reactions in range and intensity might be said to offer a fair parallel to the life of some short-cycle seed-plants, in which any substance or combination of substances which might furnish the basis for the recurrent action might be re-

newed by metabolism instead of being furnished as in the experiments described.

Of the various suggestions which might be offered in explanation of the reaction, the most plausible one seems to be one in which it is assumed that the replacement of the glycocoll solution would result in the formation of a theoretically possible glycocoll agarate, the bulk of which might be greater than the total of its separate components, and hence the combination would result in an immediate further swelling. The slow diffusion of this substance out of the sections would lessen or prevent the absorption or penetration of the glycocoll solution from the outside so a shrinkage would result. In the case of the hydroxides and mixtures containing gelatine the combination most probably would be that in which a potassium gelatinate would be formed and its slow diffusion would be accompanied by a shrinkage. In any case the changes in volume seem to have escaped observation hitherto and to be of such range as to have significance for the mechanism of the cell.

STRUCTURE AND HETEROTROPIC SWELLING OF COLLOIDAL MIXTURES.

That dried sections of colloids do not show equivalent expansion in all directions due to the development of structure in disiccation has long been known and has been variously discussed in previous articles. Extreme differentiation is shown by agar, plates of which may be so dried that they increase but 2-4 per cent. in length and width while swelling 3,000-4,000 per cent. in thickness. Gelatine on the other hand may be cast in such form that it increases 10 to 60 per cent. in length and width while hydrating 1,000 to 3,000 per cent. in thickness. Mixtures of agar and gelatine do not show more than 10-16 per cent. increase in superficial measurements. No accurate measurements have been made, but the data in Tables I. and II. suggest that the swelling in the axes in which the colloid did not shrink when desiccating may be modied in a distinct manner characteristic of the substances acting upon the colloid. Thus the relative increase in volume is greater in a mixture of 1 part agar and 3 of gelatine in hydroxide than it is in acid, and other differentiations may be found by inspection of these tables.

The superposed effects of alternated solutions may also be taken to rest partly upon the complex structure of pentosan-protein compounds. The replacement of one swelling reagent by another of higher effect on agar does not have the effect of inducing a total hydration of agar in excess of that which might be induced by the second solution applied at the beginning. On the other hand the replacement of a solution which might produce a maximum hydration by another which has a lesser effect does not usually result in reducing the water content of the colloidal mass to the amount which it would have taken up if swelled in this reagent from the beginning.

The mixture of agar and gelatine however results in a condition or structure in the colloidal mass in which it is possible to produce or secure superposed effects. So far the only available example of this action was a case in which an agar-gel mixture was first hydrated to full capacity in histidine dihydrochloride, making an increase of 920 per cent.; the amino salt was now replaced with potassium hydroxide, making a total swelling of 2,950–3,447 per cent. as compared with 2,556–3,090 per cent. which takes place in the hydroxide alone.

If the mass be supposed to be made up of alternating strands or globules of the carbohydrate and albuminous elements, it is clear that the action of the salt to which the sections were first exposed would result in a much greater hydration of the gelatine than of the agar. Replacement with the hydroxide would not result in the reduction of the salt induced swelling but would increase it at the same time facilitating hydration of the agar.

⁶ MacDougal, D. T., "Hydration and Growth," Publ. No. 297 Carnegie Inst. of Wash., 1920, see pp. 17–20.